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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12N 7/00, C12P 21/02 A61K 39/12	**A1**	(11) International Publication Number: **WO 93/03145** (43) International Publication Date: 18 February 1993 (18.02.93)

(21) International Application Number: PCT/US92/06100

(22) International Filing Date: 22 July 1992 (22.07.92)

(30) Priority data:
736,254	26 July 1991 (26.07.91)	US
918,311	21 July 1992 (21.07.92)	US

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(81) Designated States: AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE).

Published
With international search report.
With amended claims.

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(54) Title: INFECTIOUS BURSAL DISEASE VIRUS RECOMBINANT POXVIRUS VACCINE

(57) Abstract

What is described is a recombinant poxvirus, such as fowlpox virus, containing foreign DNA from infectious bursal disease virus. What is also described is a vaccine containing the recombinant poxvirus for inducing an immunological response in a host animal inoculated with the vaccine.

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INFECTIOUS BURSAL DISEASE VIRUS
RECOMBINANT POXVIRUS VACCINE

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of
5   copending application Serial No. 07/736,254 filed July
26, 1991, incorporated herein by reference. Reference is
also made to copending application Serial No. 07/847,951,
filed March 6, 1992, also incorporated herein by
reference.

10   FIELD OF THE INVENTION

The present invention relates to a modified
poxvirus and to methods of making and using the same.
More in particular, the invention relates to recombinant
poxvirus, which virus expresses gene products of an
15   infectious bursal disease virus (IBDV) gene, and to
vaccines which provide protective immunity against IBDV
infections.

Several publications are referenced in this
application. Full citation to these documents is found
20   at the end of the specification preceding the claims.
These documents pertain to the field of this invention;
and, each of the documents referenced in this application
are hereby incorporated herein by reference.

BACKGROUND OF THE INVENTION

25   Vaccinia virus and more recently other
poxviruses have been used for the insertion and
expression of foreign genes. The basic technique of
inserting foreign genes into live infectious poxvirus
involves recombination between pox DNA sequences flanking
30   a foreign genetic element in a donor plasmid and
homologous sequences present in the rescuing poxvirus
(Piccini et al., 1987).

Specifically, the recombinant poxviruses are
constructed in two steps known in the art and analogous
35   to the methods for creating synthetic recombinants of the
vaccinia virus described in U.S. Patent Nos. 5,110,587,
4,769,330, 4,722,848, and 4,603,112; the disclosures of

each of these patents is incorporated herein by
reference. Reference is also made to copending
application Serial No. 07/537,890, filed June 14, 1990,
also incorporated herein by reference.

5   First, the DNA gene sequence to be inserted
into the virus, particularly an open reading frame from a
non-pox source, is placed into an *E. coli* plasmid
construct into which DNA homologous to a section of DNA
of the poxvirus has been inserted. Separately, the DNA

10   gene sequence to be inserted is ligated to a promoter.
The promoter-gene linkage is positioned in the plasmid
construct so that the promoter-gene linkage is flanked on
both ends by DNA homologous to a DNA sequence flanking a
region of pox DNA containing a nonessential locus. The

15   resulting plasmid construct is then amplified by growth
within *E. coli* bacteria (Clewell, 1972) and isolated
(Clewell et al., 1969; Sambrook et al., 1989).
           Second, the isolated plasmid containing the DNA
gene sequence to be inserted is transfected into a cell

20   culture, e.g. chick embryo fibroblasts, along with the
poxvirus. Recombination between homologous pox DNA in
the plasmid and the viral genome respectively gives a
poxvirus modified by the presence, in a nonessential
region of its genome, of foreign DNA sequences. The term

25   "foreign" DNA designates exogenous DNA, particularly DNA
from a non-pox source, that codes for gene products not
ordinarily produced by the genome into which the
exogenous DNA is placed.
           Genetic recombination is in general the

30   exchange of homologous sections of DNA between two
strands of DNA. In certain viruses RNA may replace DNA.
Homologous sections of nucleic acid are sections of
nucleic acid (DNA or RNA) which have the same sequence of
nucleotide bases.

35   Genetic recombination may take place naturally
during the replication or manufacture of new viral
genomes within the infected host cell. Thus, genetic

recombination between viral genes may occur during the viral replication cycle that takes place in a host cell which is co-infected with two or more different viruses or other genetic constructs. A section of DNA from a

5   first genome is used interchangeably in constructing the section of the genome of a second co-infecting virus in which the DNA is homologous with that of the first viral genome.

However, recombination can also take place

10 between sections of DNA in different genomes that are not perfectly homologous. If one such section is from a first genome homologous with a section of another genome except for the presence within the first section of, for example, a genetic marker or a gene coding for an

15 antigenic determinant inserted into a portion of the homologous DNA, recombination can still take place and the products of that recombination are then detectable by the presence of that genetic marker or gene in the recombinant viral genome.

20   Successful expression of the inserted DNA genetic sequence by the modified infectious virus requires two conditions. First, the insertion must be into a nonessential region of the virus in order that the modified virus remain viable. The second condition for

25 expression of inserted DNA is the presence of a promoter in the proper relationship to the inserted DNA. The promoter must be placed so that it is located upstream from the DNA sequence to be expressed.

The technology of generating vaccinia virus

30 recombinants has recently been extended to other members of the poxvirus family which have a more restricted host range. The avipox virus, fowlpox, has been engineered as a recombinant virus. This recombinant virus is described in PCT Publication No. WO89/03429, also incorporated

35 herein by reference.

Fowlpox virus (FPV) has advantageously been engineered as a vector expressing antigens from poultry

pathogens. The hemagglutinin protein of a virulent avian influenza virus was expressed in an FPV recombinant (Taylor et al., 1988). After inoculation of the recombinant into chickens and turkeys, an immune response

5   was induced which was protective against either a homologous or heterologous virulent influenza virus challenge (Taylor et al., 1988). In addition, the surface glycoproteins (fusion and hemagglutinin) of a virulent strain of Newcastle Disease Virus have been

10 expressed in an FPV vector and shown to induce a protective immune response (Taylor et al., 1990; Edbauer et al., 1990).

FPV is the prototypic virus of the Avipox genus of the Poxvirus family. The virus causes an economically

15 important disease of poultry which has been well controlled since the 1920's by the use of live attenuated vaccines. Replication of the avipox viruses is limited to avian species (Matthews, 1982) and there are no reports in the literature of the virus causing a

20 productive infection in any non-avian species including man. This host restriction provides an inherent safety barrier to transmission of the virus to other species and makes use of FPV as a vaccine vector in poultry an attractive proposition.

25   Infectious bursal disease, also known as Gumboro's disease, manifests itself in two ways. In chickens older than three weeks, infectious bursal disease virus (IBDV) can cause impaired growth and mortality losses of up to 20% (Lukert and Hitchner,

30 1984). In younger birds, the disease is subclinical but is evident as microscopic lesions in the bursa of Fabricius (Winterfield et al., 1972). This results in prolonged and severe immunosuppression which causes increased susceptibility to disease and interferes with

35 vaccination programs against other disease agents (Allan et al., 1972). Characteristics of the disease have been

reviewed in Lukert and Saif (1991) and will be summarized briefly here.

The cloacal bursa appears to be the primary target organ of the virus and birds surgically

5   bursectomized at 4 weeks have been shown to survive a lethal IBDV challenge without clinical manifestations (Kaufer and Weis, 1980). The age of bursectomy is however, critical. Schat et al., (1981) performed embryonal bursectomy and then challenged with IBDV at 2

10   and 6 weeks of age. Birds developed typical hemorrhagic lesions, were clinically ill and showed some mortality. The target cells are actively dividing B lymphocytes (Muller, 1986; Burkhardt and Muller, 1987). Muller (1986) demonstrated that IBDV will replicate

15   preferentially in lymphoid cells from the bursa and poorly in lymphoid cells from other organs. It has been proposed that clinical signs of IBDV infection may result from immune complex formation (Ley et al., 1979; Skeeles et al., 1979). Muller (1986) however, demonstrated that

20   the preferential replication in the lymphoid cells of the bursa is not related to the presence of surface immunoglobulins.

Two serotypes of IBDV, designated 1 and 2 have been demonstrated (McFerran et al., 1980; Jackwood et

25   al., 1984; McNulty and Saif, 1988). Virulent serotypes have been shown in Group 1. No disease has been associated with group 2 viruses. In addition, considerable antigenic variation has been documented within serotypes (Lukert and Saif, 1991).

30   The causative agent, IBDV, has been classified as a Birnavirus (Brown et al., 1986). The biochemistry and replication of IBDV has been reviewed in Kibenge et al., (1988). Birnaviruses are small non-enveloped animal viruses having two segments of double-stranded RNA. The

35   smaller genomic segment (segment B) of IBDV encodes a single polypeptide of 90k designated VP1. This protein is a minor internal component of the virion and is

presumed to be the viral RNA polymerase (Hudson et al.,
1986; Nagy et al., 1987; Spies et al., 1987). The larger
genomic segment (segment A) encodes 5 polypeptides with
the following designations and approximate molecular
5   weights 52k (VPX), 41k (VP2), 32k (VP3), 28k (VP4) and
16k (Azad et al., 1985). The identity and presence of
the 16K polypeptide has not been confirmed (Kibenge et
al., 1988). VP2, VP3 and VP4 arise by co-translational
proteolytic cleavage of precursor polyproteins. The
10   protein VP4 is thought to be a viral protease (Hudson et
al., 1986) responsible for cleavage between VPX and VP4
(Duncan et al., 1987) and between VP4 and VP3 (Azad et
al., 1987; Jagadish et al., 1988).

Protein VP2 is the most abundant protein of the
15   viral capsid making up 51% of serotype I IBDV proteins
(Dobos et al., 1979). VP2 is only found in mature viral
particles and is not seen in IBDV infected cells (Becht
et al., 1988). VP2 is thought to be a specific cleavage
product of a VPX precursor. Peptide mapping has shown
20   that VPX and VP2 of IBDV strain CU-1 have similar amino
acid sequences (Muller and Becht, 1982; Dobos, 1979). In
addition both VPX and VP2 react with the same monoclonal
antibody on Western blots (Fahey et al., 1985b; Becht et
al., 1988). It has recently been demonstrated that a
25   conformational dependent neutralizing epitope exists on
VP2 (Azad et al., 1987; Fahey et al., 1989) and a
conformation independent neutralizing epitope exists on
VP3 (Fahey et al., 1985 a,b). Antibodies to these
epitopes were found to passively protect chickens (Fahey
30   et al., 1985b; Azad et al., 1987; Fahey et al. 1989).
Becht et al., (1988) and Snyder et al., (1988) indicated
that neutralizing monoclonal antibodies to VP2
differentiated between serotypes 1 and 2 in cross-
neutralization tests. However, Becht et al., (1988) also
35   indicated that monoclonal antibodies to VP3 recognized a
group-specific antigen from both serotypes which was not
associated with neutralizing activity or protection.

These studies may indicate the existence of multiple epitopes at least on VP2 and perhaps on VP3.

In a recent publication, Macreadie et al., (1990) demonstrated the expression of VP2 in a yeast

5   vector. The size of the expressed protein was consistent with that of an authentic VP2. Centrifugation and gel filtration studies indicated that the VP2 expressed in yeast was in a high molecular weight aggregated form. Chickens inoculated with a crude extract of the yeast

10 expressed VP2 developed an immune response as demonstrated by ELISA test and virus neutralization tests. One day old chickens were then inoculated with anti-sera from chickens previously inoculated with yeast expressed VP2. These chickens were passively protected

15 against IBDV challenge as evidenced by lack of IBDV antigen in the bursa (Macreadie et al., 1990).

Current vaccination strategies against IBDV include both live and killed vaccines. Antibody transmitted from the hen via the yolk of the egg can

20 protect chickens against early infections with IBDV. Therefore, use of killed vaccines in oil emulsions to stimulate high levels of maternal antibody is extensive in the field (Lukert and Saif, 1991). Studies by Lucio and Hitchner (1979) and Baxendale and Lutticken (1981)

25 indicated that oil emulsion IBDV vaccines can stimulate adequate maternal immunity to protect chickens for 4-6 weeks. In contrast progeny from breeders vaccinated with live vaccines are protected for only 1-3 weeks after hatching (Lukert and Saif, 1991).

30   Determination of when maternal antibody has waned, and thus when antibody levels can be boosted by active immunization is problematical. It is therefore common practice to vaccinate all chicks against IBD with a live vaccine during the first 3 weeks of life

35 (Winterfield et al., 1980). Inactivated vaccines are ineffective in inducing active immunity in chicks with maternal antibody. Presently available live vaccines

consist of strains of intermediate virulence or highly
attenuated strains, as well as some cell culture adapted
variant strains. While intermediate strains can break
through maternal antibody titers of approximately 1:250

5   (Lukert and Saif, 1991), the strains vary in virulence
and can induce bursal atrophy and immunosuppression in
day old and 3 week old SPF chickens (Lukert and
Mazariegos, 1985).

          Given the limitations of current vaccination

10 strategies, it can be appreciated that provision of an
IBDV recombinant poxvirus, and of vaccines which provide
protective immunity against IBDV infections, would be a
highly desirable advance over the current state of
technology.

15   OBJECTS OF THE INVENTION
          It is therefore an object of this invention to
provide recombinant poxviruses, which viruses express
gene products of IBDV, and to provide a method of making
such recombinant poxviruses.

20   It is an additional object of this invention to
provide for the cloning and expression of IBDV coding
sequences, particularly sequences coding for IBDV
structural proteins, in a poxvirus vector, particularly
fowlpox virus.

25   It is another object of this invention to
provide a vaccine which is capable of eliciting IBDV
antibodies and protective immunity against IBDV
infection.

          These and other objects and advantages of the

30 present invention will become more readily apparent after
consideration of the following.

                   STATEMENT OF THE INVENTION
          In one aspect, the present invention relates to
a recombinant poxvirus containing therein a DNA sequence

35 from IBDV in a nonessential region of the poxvirus
genome. The poxvirus is advantageously an avipox virus,
such as fowlpox virus.

According to the present invention, the
recombinant poxvirus expresses gene products of the
foreign IBDV gene. In particular, the foreign DNA codes
for IBDV structural proteins. The IBDV gene may be co-
5   expressed with other foreign genes in the host by the
recombinant poxvirus.

In another aspect, the present invention
relates to a vaccine for inducing an immunological
response in a host animal inoculated with the vaccine,
10 said vaccine including a carrier and a recombinant
poxvirus containing, in a nonessential region thereof,
DNA from IBDV. Advantageously, the DNA codes for and
expresses IBDV structural proteins. The IBDV gene may be
co-expressed with other foreign genes in the host. The
15 poxvirus used in the vaccine according to the present
invention is advantageously an avipox virus, such as
fowlpox virus, referred to hereafter as TROVAC.

BRIEF DESCRIPTION OF THE DRAWINGS

A better understanding of the present invention
20 will be had by referring to the accompanying drawings, in
which:

FIG. 1 (SEQ ID NO:7) shows the nucleotide
sequence of a 3661 base pair fragment of TROVAC DNA
containing the F8 open reading frame; and
25   FIG. 2 (SEQ ID NO:12) shows the nucleotide
sequence of a 3659 base pair fragment of TROVAC DNA
containing the F8 open reading frame.

DETAILED DESCRIPTION OF THE INVENTION

The invention is directed to recombinant
30 poxviruses containing therein a DNA sequence from IBDV in
a nonessential region of the poxvirus genome. The
recombinant poxviruses express gene products of the
foreign IBDV gene. In particular, IBDV genes encoding
IBDV structural proteins were isolated, characterized and
35 inserted into TROVAC (FPV) recombinants.

Cell Lines and Virus Strains. The strain of
FPV designated FP-1 has been previously described (Taylor

et al., 1988). It is an attenuated vaccine strain useful in vaccination of day old chickens. The parental virus strain Duvette was obtained in France as a fowlpox scab from a chicken. The virus was attenuated by

5   approximately 50 serial passages in chicken embryonated eggs followed by 25 passages on chicken embryo fibroblast (CEF) cells. This virus was obtained in September 1980 by Rhone Merieux, Lyon, France and a master viral seed established. Subsequently, the virus was subjected to

10   four successive plaque purifications. One plaque isolate was further amplified in primary CEF cells, and a stock virus, designated as TROVAC, established.

cDNA clones from IBDV strain Faragher (Type I) were obtained from Rhone Merieux, Lyon, France.

15   Example 1   -   CONSTRUCTION OF INSERTION VECTOR FOR
                      IBDV-VP2

Plasmid pIBDVA contains a 3.1 Kb KpnI to XbaI fragment derived from cDNA clones of IBDV strain Faragher. This fragment was inserted into vector

20   pBluescript II SK+ (Stratagene, La Jolla, CA). The insert corresponds to the segment A of the IBDV genome which encodes the 108 kDa precursor polyprotein. The polyprotein is subsequently processed to form VP2, VP3 and VP4.

25   In order to isolate the coding sequence for VP2 from pIBDVA, VP3 and VP4 coding sequences were deleted from pIBDVA and a termination codon added to the 3′ end of the VP2 coding sequence. This was accomplished by digestion of pIBDVA with ScaI and KpnI and insertion of

30   the annealed and kinased oligonucleotides CE279 (SEQ ID NO:1) and CE280 (SEQ ID NO:2) to form pCEN112.
      CE279

          ACTTCATGGAGGTGGCCGACCTCAACTCTCCCCTGAAGATTGCAGGAGCATT
          TGGCTTCAAAGACATAATCCGGGCTATAAGGAGGTGAGTCGACGGTAC

35   CE280

          CGTCGACTCACCTCCTTATAGCCCGGATTATGTCTTTGAAGCCAAATGCTCC
          TGCAATCTTCAGGGGAGAGTTGAGGTCGGCCACCTCCATGAAGT

The vaccinia virus H6 promoter previously
described in Taylor et al., (1988); Guo et al., (1989),
Perkus et al., (1989), was inserted into pCEN112 by
digesting pCEN112 with NotI, and blunt-ending with the
5   Klenow fragment of DNA polymerase, in the presence of 10
mM dNTPs.   A HindIII to EcoRV fragment which contains the
H6 promoter was blunt-ended with the Klenow fragment of
DNA polymerase and inserted into the linearized pCEN112
to generate pCEN117.
10   In order to couple the promoter sequence with
the initiating ATG of IBDV VP2 coding sequence, the
annealed and kinased oligonucleotides CE277 (SEQ ID NO:3)
and CE278 (SEQ ID NO:4) were inserted into pCEN117 that
had been digested with NruI and RsrII.   The resulting
15   plasmid was designated pCEN120.
CE277

     CGATATCATGACAAACCTGCAAGATCAAACCCAACAGATTGTTCCGTTCATA
     CGGAGCCTTCTGATGCCAACAACCG
CE278
20   GTCCGGTTGTTGGCATCAGAAGGCTCCGTATGAACGGAACAATCTGTTGGGT
     TTGATCTTGCAGGTTTGTCATGATATCG
          A SmaI to SalI fragment from pCEN120,
containing IBDV-VP2 linked to the vaccinia virus H6
promoter was cloned into the HpaI and SalI sites of the
25   FPV insertion vector pCEN100 (described below) to
generate pCEN137.   Plasmid pCEN137 was used in an in
vitro recombination test to generate recombinant vFP115.
Example 2   -   CONSTRUCTION OF INSERTION VECTOR FOR IBDV
                    VP2, VP3, VP4
30   Non-coding sequence was removed from the 3' end
of the IBDV polyprotein sequence by partially digesting
pIBDVA with PpuMI, completely digesting with KpnI, and
re-inserting the annealed and kinased oligonucleotides
CE275 (SEQ ID NO:5) and CE276 (SEQ ID NO:6) into pIBDVA
35   to generate pCEN111.
CE275:   GACCTTGAGTGAGTCGACGGTAC
CE276:   CGTCGACTCACTCAAG

A perfect 5' end to the polyprotein sequence
was obtained in the following manner. A KpnI-BstEII
fragment containing the majority of the polyprotein
sequence with a perfect 3' end was excised from pCEN111
5   and ligated into the KpnI and BstEII sites of pCEN120.
This substitution replaces the 3' end of the VP2 coding
sequence and generates a perfect 5' end for the
polyprotein with linkage to the vaccinia virus H6
promoter. The resulting plasmid was designated pCEN125.
10 The final insertion plasmid was constructed by partial
digestion of pCEN125 with SmaI and complete digestion
with SalI. The resulting fragment was cloned into the
HpaI and SalI sites of pCEN100 (described below) to form
pCEN138. Plasmid pCEN138 was used in an *in vitro*
15 recombination test to generate recombinant vFP116.

Example 3 –   CONSTRUCTION OF FOWLPOX INSERTION PLASMID
AT F8 LOCUS

Plasmid pRW731.15 contains a 10 Kbp PvuII-PvuII
fragment cloned from TROVAC genomic DNA. The nucleotide
20 sequence was determined on both strands for a 3661 bp
PvuII-EcoRV fragment. This sequence is shown in Figure 1
(SEQ ID NO:7). The limits of an open reading frame
designated in this laboratory as F8 were determined
within this sequence.

25   Subsequently, the nucleotide sequence of Fig. 1
was further analyzed and was determined on both strands
to be a 3659 bp PvuII-EcoRV fragment. This sequence is
shown in Figure 2 (SEQ ID NO:12). The limits of the open
reading frame designated in this laboratory as F8 were
30 determined within this sequence; and, the subsequent
determination of the sequence, as shown in Fig. 2, does
not affect the reproducibility of this or any other
construction involving the fowlpox F8 locus determined by
this laboratory, especially because the deletions and
35 insertions into the F8 ORF can be performed by the
skilled artisan following the teachings from this
laboratory, such as the following description, without

recourse to the sequence of the F8 ORF or the PvuII-EcoRV
fragment within which it is contained. Based on sequence
information contained in Figure 2, the open reading frame
is initiated at position 495 and terminates at position

5   1887. A deletion was engineered from what was ultimately
determined to be position 779 to position 1926, as
described below.

        Plasmid pRW761 is a sub-clone of pRW731.15
containing a 2430 bp EcoRV-EcoRV fragment. Plasmid

10   pRW761 was completely digested with XbaI and partially
digested with SspI. A 3700 bp XbaI-SspI band was
isolated and ligated with the annealed double-stranded
oligonucleotides JCA017 (SEQ ID NO:8) and JCA018 (SEQ ID
NO:9).

15   JCA017 5' CTAGACACTTTATGTTTTTTAATATCCGGTCTTAAAAGCTTCCCGGG
             GGATCCTTATACGGGGAATAAT 3'
      JCA018 5' ATTATTCCCCGTATAAGGATCCCCCGGGAAGCTTTTAAGACCGGATA
             TTAAAAAACATAAAGTGT 3'
        The plasmid resulting from this ligation was

20   designated pJCA002.

        Additional cloning sites were incorporated into
pJCA002 by inserting the annealed and kinased
oligonucleotides CE205 (SEQ ID NO:10) and CE206 (SEQ ID
NO:11) into the BamHI and HindIII sites of pJCA002 to

25   form pCE72.
      CE205:   GATCAGAAAAACTAGCTAGCTAGTACGTAGTTAACGTCGACCTGCAG
                AAGCTTCTAGCTAGCTAGTTTTTAT
      CE206:   AGCTATAAAAACTAGCTAGCTAGAAGCTTCTGCAGGTCGACGTTAAC
                TACGTACTAGCTAGCTAGTTTTTCT

30   In order to increase the length of the FPV
flanking arms in the insertion plasmid, plasmid pJCA021
was constructed. Plasmid pJCA021 was obtained by
inserting a 4900bp PvuII-HindII fragment from pRW731.15
(described above) into the SmaI and HindII sites of

35   pBluescript SK+ (Stratagene, La Jolla, CA). A BglII to
EcoRI fragment from pCEN72 was then ligated into the
BglII and EcoRI sites of pJCA021 to generate pCEN100.

Example 4   -   DEVELOPMENT OF TROVAC-IBDV RECOMBINANTS
        Plasmids pCEN137 and pCEN138 were transfected
into TROVAC infected primary CEF cells by using the
calcium phosphate precipitation method previously
5 described (Panicali and Paoletti, 1982; Piccini et al.,
1987). Positive plaques were selected on the basis of
hybridization to specific IBDV radiolabeled probes and
subjected to five sequential rounds of plaque
purification until a pure population was achieved. One
10 representative plaque from each IVR was then amplified
and the resulting TROVAC recombinants were designated
vFP115 (IBDV-VP2) and vFP116 (IBDV-VP2, VP3, VP4).
        Immunofluorescence. In order to determine
where the IBDV proteins were localized in recombinant
15 infected CEF cells, immunofluorescence analysis was
performed. Indirect immunofluorescence was performed was
performed as described in Taylor et al., (1990) using a
neutralizing monoclonal antibody preparation specific for
VP2 and designated AC6 and and a VP3 specific monoclonal
20 antibody designated NA3 obtained from Rhone Merieux. In
addition, a polyclonal chicken anti-IBDV serum was
obtained from Spafas Inc., Storrs, CT.
        The results indicated that IBDV specific
immunofluorescence could be detected in the cytoplasm of
25 cells infected with either vFP115 or vFP116. No
fluorescence was detected in parental TROVAC infected CEF
cells. No surface fluorescence was detected in cells
infected with either recombinant virus. Equivalent
results were obtained using both the neutralizing
30 monoclonal antibody preparation and the polyclonal immune
serum. The result was not unexpected since the analysis
of the sequence of the IBDV genes does not indicate the
presence of characteristic signal and anchor sequences
which would direct insertion of the proteins in the
35 infected cell membrane.
        Immunoprecipitation. Immunoprecipitation
reactions were performed as described in Taylor et al.,

(1990) using the monoclonal antibody preparations and the polyclonal anti-IBDV immune serum from chickens as described above.

Immunoprecipitation analysis of CEF cells
5   infected with recombinant vFP115 indicated the expression of a protein of approximately 38-40 Kd recognized by both polyclonal immune sera and the neutralizing monoclonal antibody. This size is appropriate for expression of the structural protein, VP2 (Azad et al., 1985).
10 Immunoprecipitation analysis of lysates of cells infected with recombinant vFP116 encoding the IBDV polyprotein, with the same serological reagents, also demonstrated expression of a single protein species of approximately 43 kd. This protein is recognized by both polyclonal
15 immune serum and the neutralizing monoclonal antibody preparation. Both the size of the protein and its recognition by the monoclonal antibody indicate that the identity of this protein may be VPX, the precursor to VP2. Although no other proteins are immunoprecipitated
20 by the polyclonal immune serum, presence of the cleaved VPX indicates that VP4, the cleavage protein is probably expressed. Since VP4 is a very minor component of the virion, it is not unusual that the immune serum should not contain antibodies to this protein. Use of the VP3
25 specific monoclonal antibody indicated the expression of a protein of 32 kd in cells infected with vFP116.
    Example 5 -   IMMUNIZATION OF CHICKENS AND SUBSEQUENT
                   CHALLENGE
         Groups of 20, 5 day old susceptible SPF
30 chickens were inoculated by subcutaneous injection in the nape of the neck with 0.2 ml of recombinants vFP115 or vFP116. This corresponded to a dose of approximately 4.0 $\log_{10}$ TCID$_{50}$. A group of 19 birds were left as uninoculated controls. At fourteen days post
35 vaccination, chickens were bled and serum neutralizing titers in the sera were determined. Birds were challenged at 14 days by intra-ocular inoculation of 0.03

ml of the virulent heterologous serotype I IBDV challenge
strain (designated STC) supplied by the USDA National
Veterinary Services Laboratory. Five days after
challenge, each chicken was necropsied and the bursa
5   examined for gross lesions and the appearance of atrophy.
The results are shown in Table 1.

The results indicate that inoculation of one
dose of vFP115 expressing the VP2 structural protein
leads to the induction of serum neutralizing antibody and
10 75% protection of challenged birds. Inoculation of
vFP116 leads to the induction of a poor neutralizing
antibody response but 50% of challenged birds are
protected.

TABLE 1.   Protective Efficacy of TROVAC-IBDV
15   Recombinants in Chickens

Recombinant	#Protected/ Challenged[b]	% Protection	SN Titer[a]
vFP115	15/20	75	131
vFP116	10/19	53	6
Controls	0/19	0	0

a: Serum neutralization titer
25 b: Birds are considered protected in the absence of
bursal atrophy and lesions.

Example 6   -   IBDV RECOMBINANT POXVIRUS VACCINES
Recombinant poxviruses containing, in a
nonessential region thereof, DNA from IBDV provide
30 advantages as vaccines for inducing an immunological
response in a host animal. Infectious bursal disease
virus is very stable and persists in the environment for
long periods. For economic reasons, poultry houses are
rarely cleaned between broods and thus chickens are
35 exposed to the virus early in life. Since elimination of
virus by hygienic means is not possible, vaccination
strategies need to be formed. Active immunization of

chickens is difficult in the presence of maternal
antibody. In addition, since maternal antibody levels
are variable and the rate of loss of antibody
unpredictable, timing of vaccination is a problem. A
5   successful vaccine will need to be able to boost immunity
in the presence of maternal antibody and should also
contain cross-reactive antigens from a number of
different serotypes. In addition, an effective vaccine
should not induce signs of disease in vaccinated birds.
10   TROVAC-IBDV recombinant vFP115 expressed the
major structural protein VP2 which has been shown to
contain at least one highly immunogenic region. The
protein expressed by the TROVAC recombinant is
recognizable by IBDV immune serum. Inoculation of this
15   recombinant into susceptible birds resulted in 75%
protection from bursal damage. Recombinant vFP116
contains the coding sequence for the polyprotein VP2,
VP3, VP4. A protein probably corresponding to VPX, the
VP2 precursor, was expressed which is also recognized by
20   IBDV immune sera. Inoculation of this recombinant into
susceptible birds lead to the development of low
neutralizing antibody levels, but induced 53% protection
from bursal damage.
             The results indicate the potential of TROVAC-
25   IBDV recombinants for vaccination against IBDV in the
poultry industry. The restricted host range of FPV
provides an inherent safety barrier to transmission of
recombinant to non-vaccinated species. Use of antigenic
regions of IBDV rather than whole virus eliminates the
30   need to introduce live virus to the environment and may
lessen the immunological pressure on the virus which
leads to the emergence of variant strains. The large
size of the FPV genome allows incorporation of multiple
antigenic sequences and should allow for vaccination
35   against a variety of strains.
     Example 7   -   FURTHER IMMUNOGENICITY AND EFFICACY
                     STUDIES WITH vFP115

<u>Effect of dose of inoculation on protective</u>
<u>efficacy induced by vFP115</u>. Groups of day old SPF
chickens were inoculated with vFP115 by the subcutaneous
route in the nape of the neck.   The virus was
5   administered in doses of 4.9, 5.5 or 6.2 $\log_{10}$ EID_{50} per
bird. At 21 days post-vaccination, ten vaccinates and
ten naive birds were bled and the sera analyzed for the
presence of IBDV specific serum neutralizing (SN)
antibody.   At 28 days, birds were challenged by
10   administration by the ocular route of 1.3 $\log_{10}$ EID_{50} of
the heterologous Standard Challenge Strain of IBDV. At 5
days post-challenge, 5 birds from each group were
necropsied and bursae examined for gross lesions. At 11
days post-challenge, the remaining birds were killed and
15   bursa to body weight ratios determined.   The results of
analysis are shown in Table 2.   The results indicate that
increasing the inoculation dose has led to the induction
of slightly higher levels of SN antibody, but that the
protective efficacy is not enhanced.   Birds were
20   considered protected when the bursa to body weight ratio
after challenge was greater than one standard deviation
of the mean bursa to body weight ratio of infected
control birds.   Using this criteria, and considering
bursa to body weight ratios of individual birds,
25   protection ratios of 65%, 74% and 64% were obtained for
vFP115 dosages of 4.9, 5.5 and 6.2 $\log_{10}$ EID_{50}
respectively.

30

35

TABLE 2.   Dose Response Study of Inoculation of vFP115 in
           Day Old Chickens

Dose	SN GMT[a]	Bursal Lesions[b] Positive/Total	Bursa/Body Weight Ratio[c]
4.9	13	1/5	3.5
5.5	35	2/5	3.2
6.2	102	1/5	3.2
Control	0	5/5	1.6

a: Geometric Mean Titer of sera of 10 birds

b: Bursa of 5 birds examined for gross lesions

c: Ratio expressed as a mean of 23 birds

Effect of Age of Bird on Protective Efficacy of
vFP115. Groups of 30 one-, four-, seven- and fourteen
day old SPF birds were inoculated by the subcutaneous
route with 4.0 $\log_{10}$ EID_{50} of vFP115. At 21 days post-
vaccination, 10 vaccinates and 5 naive controls of each
group were bled and sera analyzed for the presence of SN
antibody. At 28 days post-vaccination, all vaccinates
and naive controls were challenged by the ocular route
with 1.3 $\log_{10}$ EID_{50} of the heterologous STC virus strain.
Four days post-challenge, birds were sacrificed and bursa
examined for evidence of bursal damage. The results of
analysis are shown in Table 3. The results indicate that
while IBD specific SN titers and protection after
challenge are obtained at one day of age, when
vaccination is delayed past 4 days of age higher SN
titers are obtained and the level of protection is
increased.

TABLE 3.   Effect of Age of Bird on Protective Efficacy of
            VFP115

Age Group	Treatment	GMT	Protection Ratio	% Protection
1 day	Vaccinates	126	23/30	77
	Controls		0/10	0
4 days	Vaccinates	666	25/30	83
	Controls		0/10	0
7 days	Vaccinates	1946	29/30	97
	Controls		1/10	0
14 days	Vaccinates	1408	30/30	100
	Controls		0/10	0

Effect of route of inoculation on induction of
a protective immune response by vFP115. Groups of twenty
14 day old SPF birds were inoculated by (a) the
intramuscular route in the leg, (b) ocular route or (c)
oral route with 4.0 $\log_{10}$ TCID$_{50}$ of vFP115. At 14 and 28
days post-inoculation sera were collected and analyzed
for the presence of IBDV specific SN antibody. At both
14 and 28 days post-vaccination, groups of birds were
challenged by ocular inoculation of 2.5 $\log_{10}$ EID$_{50}$ of the
homologous Faragher strain of IBDV. Deaths were recorded
and at 4 days post-challenge all birds were sacrificed
and Bursa examined for the presence of macroscopic
lesions. Significant neutralizing antibody responses
were found only after inoculation of vFP115 by the
intramuscular route with SN titers of approximately 2.0
$\log_{10}$ at 14 and 28 days post-inoculation. By ocular and
oral routes, low SN titers were achieved in 30 and 10% of
chickens, respectively. The results of challenge are
shown in Table 4. All birds inoculated with vFP115 by
the intramuscular route were fully protected from
challenge which was pathogenic in all control non-
vaccinated birds at 14 and 28 days post-inoculation.   No

21

protection was observed following the oral route of
inoculation.   Partial protection was seen by the ocular
route.

TABLE 4.   <u>Effect of Route of Inoculation on Protective</u>
5   <u>Efficacy Induced By vFP115</u>

Route of	% Protection from challenge at	
Inoculation	14 days post-vacc	28 days post-vacc
Intramuscular	100	100
Ocular	50	10
Oral	0	0

<u>Example 8</u>   —   <u>DEVELOPMENT OF A TROVAC RECOMBINANT</u>
15   <u>EXPRESSING THE VP3 STRUCTURAL PROTEIN</u>

        Example 2 describes the development of a TROVAC
based recombinant vFP116 expressing the VP2, VP4, VP3
polyprotein.   Efficacy studies described in Example 5
indicate that this recombinant induces lower levels of
20 protection than vFP115 expressing the VP2 protein after
inoculation into susceptible chickens.   *In vitro* studies
showed that the VP2 protein expressed in the vFP116
construct is slightly larger than that expressed in the
vFP115 construct and that expression of the VP3 protein
25 is not detectable by a polyclonal serum.
Immunofluorescence and immunoprecipitation analysis with
a VP3 specific monoclonal antibody, however, indicated
that the VP3 protein is expressed in vFP116.   In order to
evaluate the role of the VP3 protein in eliciting cross-
30 protective immunity, a single recombinant was developed
expressing the VP3 protein from the Faragher strain of
IBDV.

        <u>Construction of a Fowlpox Insertion Plasmid at</u>
<u>the F16 Locus</u>.   The plasmid pFP23K (described by
35 Tartaglia et al., 1990) contains a 10.5 kb <u>Hind</u>III
fragment from the fowlpox (FP) genome.   A 7.3 kb
<u>Nae</u>I\<u>Nde</u>I FP fragment was isolated from pFP23K and

ligated to a similarly cut pUC9 vector to generate
pRW866. A unique FspI site within this FP fragment lies
between two ORFs (intergenic region) and is the F16
insertion locus.

5   In order to create a multiple cloning site
(MCS) cassette for the F16 locus, two PCR fragments were
amplified from pFP23K using primers RW264 (SEQ ID NO:13)
plus RW265 (SEQ ID NO:14) and RW266 (SEQ ID NO:15) plus
RW267 (SEQ ID NO:16). The resulting fragments were mixed
10 together and amplified with primers RW266 and RW267 which
resulted in a single, fused fragment. This fragment was
digested with EcoRI and NdeI and ligated into similarly
cut pRW715 (derived from pUC9 by digesting with PvuII and
ligating an EcoRI linker between the two PvuII sites), to
15 yield pRW864. The MCS cassette consists of a polycloning
region (SmaI-BamHI-HindIII sites) flanked on either side
by translational stop codons in all six reading frames
and a NotI site. A vaccinia early transcriptional stop
signal is located on the HindIII end.

20 RW264:   AATTAACCCGGGATCCAAGCTTCTAGCTAGCTAATTTTT
            ATAGCGGCCGCTATAATCGTTAACTTATTAG

    RW265:   CTAGCTAGAAGCTTGGATCCCGGGGTTAATTAATTAATAAAAA
            GCGGCCGCGTTAAAGTAGAAAAATG

    RW266:   GTTACATATGTACAGAATCTGATCATAG

25 RW267:   GCTAGAATTCTCTTAGTTTTTATAGTTG

          The following describes a series of plasmid
constructs which ultimately leads to the MCS cassette
from pRW864 being inserted into the FspI site of pRW866
to generate the F16 insertion plasmid (pRW873). A
30 cassette containing the E. coli lacZ gene coupled to the
vaccinia 11K promoter was excised from pAM1BG as a
BamHI/PstI fragment. Plasmid pAM1BG contains the lacZ
BamHI fragment from pMC1871 (Casadaban et al., 1983)
inserted in the previously described BamHI site 3' of the
35 11K vaccinia virus promoter (Paoletti et al., 1984). The
ends were repaired using Klenow polymerase and the
cassette ligated into pRW864 cut with SmaI to yield

pRW867A. The lacZ gene cassette was excised from pRW867A using NotI and the ends repaired with Klenow polymerase. This fragment was then ligated into the unique FspI site in the FP sequences of pRW866 resulting in pRW868. The

5   lacZ gene from pRW868 was excised using NotI and replaced with the MCS cassette derived as a NotI fragment from pRW864 resulting in pRW873, the F16 insertion plasmid.

Development of an FP recombinant expressing VP3. The complete IBDV VP3 ORF was excised from pCEN111

10   (described in Example 3) as a 1262 bp BamHI and Asp718 fragment and ligated into a similarly cut pSD554VC (a vaccinia donor plasmid containing the H6 promoter) to yield pFT1. A 112 bp PCR fragment was amplified from pCEN111 using oligonucleotides JP003 (SEQ ID NO:17) and

15   JP004 (SEQ ID NO:18), digested with NruI/ScaI, and gel purified. This fragment was ligated into pFT1 digested completely with NruI and partially with ScaI to yield pIBDV-VP3II. This plasmid contains the vaccinia H6 promoter coupled to the VP3 ORF.

20   A PCR fragment was amplified from pRW823 which contains vaccinia virus H6 promoter sequences using oligonucleotides RG662 (SEQ ID NO:19) and RG663 (SEQ ID NO:20). This fragment was digested with HindIII/SmaI and ligated into the F16 insertion plasmid (pRW873) cut with

25   the same enzymes resulting in pF16VQH6. A cassette containing part of the H6 promoter fused to the VP3 ORF was excised from pIBDV-VP3II with NruI/Asp718, the ends repaired with Klenow polymerase, and the purified fragment ligated into pF16VQH6 cut with NruI/SmaI to

30   generate the donor plasmid pF16VP3F.

JP003   5'-AAGGTAGTACTGGCGTCC-3'

JP004   5'-TTATCGCGATATCCGTTAAGTTTGTATCGTAATATGTTCCCTCACA-
           ATCCACGA-3'

RG662   5'-TAAAAGCTTTTAATTAATTAGTCATC-3'

35   RG663   5'-TAACCCGGGCGATACAAACTTAACGG-3'

        Plasmid pF16VP3F was used in *in vitro* recombination with TROVAC as the rescuing virus to derive

recombinant vFP186. Immunoprecipitation analysis using a
VP3 specific monoclonal antibody has confirmed the
expression of a protein of approximately 32 kd in CEF
cells infected with the recombinant.

5 Example 9 -   DEVELOPMENT OF TROVAC BASED RECOMBINANTS
                   WITH ALTERED MODES OF EXPRESSION OF THE
                   VP2 PROTEIN

It has been postulated that a protein displayed
on the infected cell surface may lead to a more efficient
10 induction of neutralizing antibody than if the protein is
secreted or expressed internally. Previous studies have
indicated that expression of a foreign antigen on the
infected cell surface by a recombinant vaccinia virus,
can be achieved by recombinant DNA techniques by adding
15 appropriate signal and anchor sequences (Langford et al.,
1986; Vijaya et al., 1988). The VP2 protein in IBDV
infected cells is not a membrane bound glycoprotein and
possesses neither an endogenous signal nor anchor
sequences. A strategy was devised to add the appropriate
20 signal and anchor sequences from the Newcastle Disease
Virus fusion protein. The fusion protein is an integral
membrane bound glycoprotein. This strategy is described
below.

The IBDV VP2 ORF plus translational stop codon
25 was excised from pCEN112 (described in Example 1) as an
XbaI/SalI fragment and the ends repaired using Klenow
polymerase. This cassette was ligated into the HincII
site of pUC18 to generate pCE147. The vaccinia H6
promoter coupled to the NDV fusion gene signal sequence
30 was obtained by isolating a HindIII/PstI fragment from
pCE64 (for complete NDV Fusion sequences see Taylor et
al., 1990). This fragment contains the H6 promoter fused
to the first 25 codons from the N-terminus of the NDV
fusion ORF. This fragment was ligated into pCE147 cut
35 with HindIII/PstI to yield pCEN150.

In order to couple the last codon from the NDV
fusion signal sequence with the first codon from the VP2

ORF, a PCR fragment was amplified from pCEN150 using
oligonucleotides CE329 (SEQ ID NO:21) and CE330 (SEQ ID
NO:22) as primers. The fragment was digested with
KpnI/RsrII and ligated into pCEN150 cut with the same
5   enzymes to generate pCEN156. The H6 promoted-NDV fusion
signal sequence-VP2 ORF cassette was excised from pCEN156
with HindIII/EcoRI, the ends repaired using Klenow
polymerase, and the cassette ligated into pCEN100 (the F8
insertion plasmid) cut with HpaI to generate the donor
10   plasmid pIBDV-VP2-SS.

CE329   5'-GATCCCGGTACCTCTAATGCTGATCATCCGAACCGCGCTGACACTG-
          AGCTGTACAAACCTGCAAGATCAAAC-3'
CE330   5'-GGACGCCGGTCCGGTTGTTGGCATC-3'

            To add the NDV fusion transmembrane sequences
15   to the above plasmid, a 240 bp PCR fragment was amplified
from pIBDV-VP2-SS using primers RG583 (SEQ ID NO:23) and
RG590 (SEQ ID NO:24). This fragment contains 49 codons
plus stop codon from the C-terminus of the NDV fusion ORF
(see Taylor et al., 1990). The purified fragment was
20   digested with ScaI/BamHI and ligated into pIBDV-VP2-SS
cut completely with BamHI and partially with ScaI to
generate the donor plasmid pIBDV-VP2-SSA.

RG583   5'-GTGAGTACTTCATGGAGGTGGCCGACCTCAACTCTCCCCTGAAGA-
          TTGCAGGAGCATTTGGCTTCAAAGACATAATCCGGGCTATAAGGA-
25   GGATCGTTTTAACTGTCATATC-3'
RG590   5'-TTAGGATCCTCATATTTTTGTAGTGGCTCTC-3'

            In vitro recombination using plasmid pIBDV-VP2-
SS and TROVAC as the rescuing virus generated recombinant
vFP147. Expression analysis of this recombinant with
30   both polyclonal immune serum and a VP2 specific
monoclonal antibody indicated that the VP2 protein is
expressed internally, and in addition is secreted into
the tissue culture fluid. This result is in keeping with
the addition of a signal sequence to the coding sequence
35   of the VP2 protein. In vitro recombination using plasmid
pIBDV-VP2-SSA and TROVAC as the rescuing virus generated
recombinant vFP151. Expression analysis using both

polyclonal immune serum and the VP2 specific monoclonal antibody indicated that the VP2 protein is expressed at the infected cell surface as expected following the addition of an anchor sequence. The fact that the VP2

5   protein is still recognized by the monoclonal antibody in this form of presentation indicates that conformation of this particular epitope has not been altered by the manipulations.

        Efficacy studies were performed by inoculating

10 day-old SPF chickens with 4.0 $\log_{10}$ $TCID_{50}$ of each recombinant. At 28 days birds were challenged by ocular inoculation of the heterologous STC challenge strain. In contrast to previous results obtained with the unmodified VP2 expressed in vFP115, no protection was obtained after

15 vaccination with either vFP147 or vFP151. Further *in vitro* studies using tunicamycin, an inhibitor of N-linked glycosylation, have indicated that the modified VP2 proteins expressed by both vFP147 and vFP151 are glycosylated whereas the unmodified VP2 expressed in

20 vFP115 is not. It is postulated that the addition of sugar moieties to the VP2 protein may alter conformation of the protein in areas apart from the neutralizing epitope. Alternatively, the addition of the signal and anchor sequences as constructed here, may alter

25 conformation of the protein. In either case it appears that the antibody induced by the modified constructions is not able to neutralize the heterologous challenge virus (STC). However, vFP147 and vFP151 and products therefrom are nonetheless useful. The modified VP2

30 expressed by these recombinants can be used as precursors to generate the VP2 protein; for example, by removal of the additional sugar moieties or to isolate secreted VP2 protein from tissue culture supernatant for further purification.

35 Example 10 - DEVELOPMENT OF POXVIRUS RECOMBINANTS
                EXPRESSING THE VP2 PROTEIN FROM
                HETEROLOGOUS STRAINS OF IBDV

IBDV strains show considerable variation in their ability to cross-neutralize. Sequence analysis of different strains has shown that one critical region involved in virus neutralization resides within a

5   conformational epitope located on VP2. Sequence information for VP2 is available for the Faragher (Bayliss et al., 1990) and STC (Kibenge et al., 1990) strains and it has been determined that five amino acid differences between the two strains occur within the

10   conformational epitope. A strategy was therefore devised to alter the coding sequence of the Faragher strain conformational epitope to conform with the sequence of the STC strain. This procedure is described below.

Mutagenesis of VP2 Faragher to VP2 STC. In

15   order to change the VP2 Faragher sequence in pCEN120 (described in Example 1) to the VP2 STC sequence, five codons were changed in the VP2 ORF using PCR site directed mutagenesis (see Kibenge et al., 1990 for STC sequence). Oligonucleotide primers RG677 (SEQ ID NO:25)

20   plus RG678 (SEQ ID NO:26) and RG685 (SEQ ID NO:27) plus RG686P (SEQ ID NO:28) were used to amplify a 530 bp and a 270 bp fragment respectively from pCEN100 (described in Example 3). The gel purified 270 bp fragment was further amplified using oligonucleotides RG702 (SEQ ID NO:29) and

25   RG704 (SEQ ID NO:30). The 530 bp fragment was digested with SacI and partially digested with PstI. The 270 bp fragment was digested with SacI and NcoI. These purified PCR amplified fragments, which contain the five STC codon changes, were ligated into pCEN120 cut with PstI and

30   NcoI. The resulting plasmid, pVP2-STC was confirmed by DNA sequencing analysis.

RG677   5'-TACACACTGCAGAGCAATGGGAACCTCAAGTTCGATCAGATG-3'

RG678   5'-GAAACACGAGCTCTCCCCCAACGCTGAGGCTTGTGATAG-3'

RG685   5'-GGAAGAGCTCGTGTTTCAAACAAGCGTCCAAGGCCTTGTACTGGG-

35   CGCCACCATCTACTTTATAGGCTTTGATGGGACTACGGTAATCAC-
         CAGAGCTGTAGCCGCAGATAATGGGCTGACGGCCGGCACCGACAA-
         TCTTATGCCATTCAATCTTG-3'

RG686P   5'-CCACCATGGATCGTCACTGCTAGGCTCCCACTTGCCGACCATGAC-
             ATCTGATCCCCTGCCTGACCACCACTTTTGGAGGTCACTACCTCC-
             AGTTTGATGGATGTGATTGGCTGGGTTATCTCATTGGTTGGAATG-
             ACAAGATTGAATGGCATAAG-3'
5 RG702   5'-GGGAGAGCTCGTGTTTCAAACAAGCG-3'
   RG704   5'-CCACCATGGATCGTCACTGC-3'

Construction of the new F8 insertion plasmid.
In order to remove all of F8 coding sequences from the
original F8 insertion plasmid (pCEN100), a new F8
10 insertion plasmid was constructed. pJCA021 contains a
4900 bp PvuII/HincII fragment from TROVAC which includes
the F8 gene and flanking sequences. A 4.2 kb NciI/PpuMI
fragment was isolated from this plasmid and the ends
repaired with Klenow polymerase. This fragment was
15 ligated into pBluescript SK+ cut with XbaI/Asp718 and
repaired with Klenow polymerase to yield pIY.
         The strategy to delete the F8 ORF from pIY and
replace it with a multiple cloning site (MCS) used PCR
amplification of two fragments from pJCA021 with
20 oligonucleotide primers containing the multiple cloning
sequences. A 335 bp fragment was amplified from pJCA021
using oligonucleotides RG714 (SEQ ID NO:31) and RG715
(SEQ ID NO:32) and digested with HindIII and EcoRI.
Similarly, a 465 bp fragment was amplified from pJCA021
25 using oligonucleotides RG716 (SEQ ID NO:33) and RG717
(SEQ ID NO:34) and digested with HindIII and BglII. The
two PCR fragments were ligated into pIY cut with EcoRI
and BglII in a three fragment ligation resulting in pF8.
This plasmid is the new F8 insertion plasmid which
30 contains a MCS consisting of SmaI, NruI, HindIII, BamHI
and XhoI sites flanked by vaccinia early transcriptional
stop signals and translational stops in all six frames.
The length of the left arm is about 1430 bp and the
length of the right arm is about 1380 bp. The F8 gene
35 ORF which initiates at nucleotide position 495 and
terminates at nucleotide position 1887 (Figure 2) is
completely deleted.

        RG714   5'-AACATATTTCCGAACAG-3'

        RG715   5'-TCCAAGCTTTCGCGACCCGGGTTTTTATTAGCTAATTAGCAATAT-
                AGATTCAATATG-3'

        RG716   5'-ATCAAGCTTGGATCCCTCGAGTTTTTATTGACTAGTTAATCATAA-
   5   GATAAATAATATACAGC-3'

        RG717   5'-GATATAGAAGATACCAG-3'

            <u>Construction of donor plasmids and recombinants</u>
<u>expressing VP2 STC</u>. A cassette containing the H6
promoted VP2 (STC) ORF was excised as a 1.5 kb <u>Sma</u>I-
10 <u>Asp</u>718 fragment from pVP2-STC. The ends were repaired
using Klenow polymerase and ligated into pF8 cut with
<u>Sma</u>I to generate the pF8-STC donor plasmid.

            Plasmid pF8-STC was used in *in vitro*
recombination with TROVAC as the rescuing virus to
15 generate recombinant vFP209. Expression analysis of the
recombinants using a polyclonal IBDV serum from chicken
indicated that the VP2 protein is expressed internally in
CEF cells infected by the recombinant.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Virogenetics Corporation

    (ii) TITLE OF INVENTION: Infectious Bursal Disease Virus
                               Recombinant Poxvirus Vaccine

    (iii) NUMBER OF SEQUENCES: 34

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        (F) ZIP: 10036

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE: 22-JUL-1992
        (C) CLASSIFICATION:

    (viii) ATTORNEY/AGENT INFORMATION:
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        (C) REFERENCE/DOCKET NUMBER: 454310-2441

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(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 100 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACTTCATGGA GGTGGCCGAC CTCAACTCTC CCCTGAAGAT TGCAGGAGCA TTTGGCTTCA   60

AAGACATAAT CCGGGCTATA AGGAGGTGAG TCGACGGTAC   100

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 96 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGTCGACTCA CCTCCTTATA GCCCGGATTA TGTCTTTGAA GCCAAATGCT CCTGCAATCT   60

TCAGGGGAGA GTTGAGGTCG GCCACCTCCA TGAAGT   96

(2) INFORMATION FOR SEQ ID NO:3:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 77 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGATATCATG ACAAACCTGC AAGATCAAAC CCAACAGATT GTTCCGTTCA TACGGAGCCT   60

TCTGATGCCA ACAACCG   77

(2) INFORMATION FOR SEQ ID NO:4:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 80 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTCCGGTTGT TGGCATCAGA AGGCTCCGTA TGAACGGAAC AATCTGTTGG GTTTGATCTT   60

GCAGGTTTGT CATGATATCG   80

(2) INFORMATION FOR SEQ ID NO:5:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 23 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GACCTTGAGT GAGTCGACGG TAC   23

(2) INFORMATION FOR SEQ ID NO:6:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 16 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGTCGACTCA CTCAAG   16

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 3661 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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GATATCTGTG GTCTATATAT ACTACACCCT ACCGATATTA ACCAACGAGT TTCTCACAAG      60

AAAACTTGTT TAGTAGATAG AGATTCTTTG ATTGTGTTTA AAAGAAGTAC CAGTAAAAAG     120

TGTGGCATAT GCATAGAAGA AATAAACAAA AAACATATTT CCGAACAGTA TTTTGGAATT     180

CTCCCAAGTT GTAAACATAT TTTTTGCCTA TCATGTATAA GACGTTGGGC AGATACTACC     240

AGAAATACAG ATACTGAAAA TACGTGTCCT GAATGTAGAA TAGTTTTTCC TTTCATAATA     300

CCCAGTAGGT ATTGGATAGA TAATAAATAT GATAAAAAAA TATTATATAA TAGATATAAG     360

AAAATGATTT TTACAAAAAT AACCTATAAG AACAATAAAA ATATAATTAC ATTTACGGAA     420

AATAGCTGGT TTTAGTTTAC CAACTTAGAG TAATTATCAT ATTGAATCTA TATTGTTTTT     480

TAGTTATATA AAAACATGAT TAGCCCCCAA TCGGATGAAA ATATAAAGA TGTTGAGAAT      540

TTCGAATACA ACAAAAGAG GAATCGTACG TTGTCCATAT CCAAACATAT AAATAAAAAT     600

TCAAAAGTAG TATTATACTG GATGTTTAGA GATCAACGTG TACAAGATAA TTGGGCTTTA     660

ATTTACGCAC AACGATTAGC GTTAAAACTC AAAATACCTC TAAGAATATG CTTTTGTGTC     720

GTGCCAAAAT TTCACACTAC TACTTCTAGT ACACTTTATG TTTTTAATAT CCGGTCTTAA     780

AGAAGTCGCG GAAGAATGTA AAAGACTATG TATAGGGTTT TCATTGATAT ATGGCGTACC     840

AAAAGTAATA ATTCCGTGTA TAGTAAAAAA ATACAGAGTC GGAGTAATCA TAACGGATTT     900

CTTTCCATTA CGTGTTCCCG AAAGATTAAT GAAACAGACT GTAATATCTC TTCCAGATAA   ·  960

CATACCTTTT ATACAAGTAG ACGCTCATAA TATAGTACCT TGTTGGGAAG CTTCTGATAA    1020

AGAAGAATAC GGTGCACGAA CTTTAAGAAA AAAGATATTT GATAAATTAT ATGAATATAT    1080

GACAGAATTT CCTGTTGTTC GTAAACATCC ATACGGTCCA TTTTCTATAT CTATTGCAAA    1140

ACCCAAAAAT ATATCATTAG ACAAGACGGT ATTACCCGTA AAATGGGCAA CGCCTGGAAC    1200

AAAAGCTGGA ATAATTGTTT TAAAAGAATT TATAAAAAAC AGATTACCGT CATACGACGC    1260

GGATCATAAC AATCCTACGT GTGACGCTTT GAGTAACTTA TCTCCGTGGC TACATTTTGG    1320

TCATGTATCC GCACAACGTG TTGCCTTAGA AGTATTAAAA TGTATACGAG AAAGCAAAAA    1380

AAACGTTGAA ACGTTTATAG ATGAAATAAT TGTAAGAAGA GAACTATCGG ATAATTTTTG    1440

TTACTATAAC AAACATTATG ATAGTATCCA GTCTACTCAT TCATGGGTTA GAAAAACATT    1500

AGAAGATCAC ATTAATGATC CTAGAAAGTA TATATATTCC ATTAAACAAC TCGAAAAAGC    1560

GGAAACTCAT GATCCTCTAT GGAACGCGTC ACAAATGCAG ATGGTGAGAG AAGGAAAAAT    1620

GCATAGTTTT TTACGAATGT ATTGGGCTAA GAAGATACTT GAATGGACTA GAACACCTGA    1680

AGACGCTTTG AGTTATAGTA TCTATTTGAA CAACAAGTAC GAACTAGACG GCACGGATCC    1740
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TAACGGATAC GTAGGTTGTA TGTGGTCTAT TTGCGGATTA CACGATAGAG CGTGGAAAGC   1800

AAGACCGATA TTTGGAAAGA TAAGATATAT GAATTATGAG AGTTCTAAGA AGAAATTTGA   1860

TGTTGCTGTA TTTATACAGA AATACAATTA AGATAAATAA TATACAGCAT TGTAACCATC   1920

GTCATCCGTT ATACGGGGAA TAATATTACC ATACAGTATT ATTAAATTTT CTTACGAAGA   1980

ATATAGATCG GTATTTATCG TTAGTTTATT TTACATTTAT TAATTAAACA TGTCTACTAT   2040

TACCTGTTAT GGAAATGACA AATTTAGTTA TATAATTTAT GATAAAATTA AGATAATAAT   2100

AATGAAATCA AATAATTATG TAAATGCTAC TAGATTATGT GAATTACGAG GAAGAAAGTT   2160

TACGAACTGG AAAAAATTAA GTGAATCTAA AATATTAGTC GATAATGTAA AAAAAATAAA   2220

TGATAAAACT AACCAGTTAA AAACGGATAT GATTATATAC GTTAAGGATA TTGATCATAA   2280

AGGAAGAGAT ACTTGCGGTT ACTATGTACA CCAAGATCTG GTATCTTCTA TATCAAATTG   2340

GATATCTCCG TTATTCGCCG TTAAGGTAAA TAAAATTATT AACTATTATA TATGTAATGA   2400

ATATGATATA CGACTTAGCG AAATGGAATC TGATATGACA GAAGTAATAG ATGTAGTTGA   2460

TAAATTAGTA GGAGGATACA ATGATGAAAT AGCAGAAATA ATATATTTGT TTAATAAATT   2520

TATAGAAAAA TATATTGCTA ACATATCGTT ATCAACTGAA TTATCTAGTA TATTAAATAA   2580

TTTTATAAAT TTTATAAATT TTAATAAAAA ATACAATAAC GACATAAAGA TATTTAATCT   2640

TTAATTCTTG ATCTGAAAAA CACATCTATA AAACTAGATA AAAAGTTATT CGATAAAGAT   2700

AATAATGAAT CGAACGATGA AAAATTGGAA ACAGAAGTTG ATAAGCTAAT TTTTTTCATC   2760

TAAATAGTAT TATTTTATTG AAGTACGAAG TTTTACGTTA GATAAATAAT AAAGGTCGAT   2820

TTTTACTTTG TTAAATATCA AATATGTCAT TATCTGATAA AGATACAAAA ACACACGGTG   2880

ATTATCAACC ATCTAACGAA CAGATATTAC AAAAAATACG TCGGACTATG GAAAACGAAG   2940

CTGATAGCCT CAATAGAAGA AGCATTAAAG AAATTGTTGT AGATGTTATG AAGAATTGGG   3000

ATCATCCTCA ACGAAGAAAT AGATAAAGTT CTAAACTGGA AAAATGATAC ATTAAACGAT   3060

TTAGATCATC TAAATACAGA TGATAATATT AAGGAAATCA TACAATGTCT GATTAGAGAA   3120

TTTGCGTTTA AAAAGATCAA TTCTATTATG TATAGTTATG CTATGGTAAA ACTCAATTCA   3180

GATAACGAAC ATTGAAAGAT AAAATTAAGG ATTATTTTAT AGAACTATT CTTAAAGACA   3240

AACGTGGTTA TAAACAAAAG CCATTACCCG GATTGGAAAC TAAAATACTA GATAGTATTA   3300

TAAGATTTTA AAAACATAAA ATTAATAGGT TTTTATAGAT TGACTTATTA TATACAATAT   3360

GGATAAAAGA TATATATCAA CTAGAAAGTT GAATGACGGA TTCTTAATTT TATATTATGA   3420

TTCAATAGAA ATTATTGTCA TGTCGTGTAA TCATTTTATA AATATATCAG CGTTACTAGC   3480

TAAGAAAAAC AAGGACTTTA ATGAATGGCT AAAGATAGAA TCATTTAGAG AAATAATAGA   3540

TACTTTAGAT AAAATTAATT ACGATCTAGG ACAACGATAT TGTGAAGAAC TTACGGCGCA   3600

TCACATTCCA GTGTAATTAT TGAGGTCAAA GCTAGTAACT TAATAGATGA CAGGACAGCT   3660

G                                                                  3661
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(2) INFORMATION FOR SEQ ID NO:8:

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        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 69 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear


        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTAGACACTT TATGTTTTTT AATATCCGGT CTTAAAAGCT TCCCGGGGGA TCCTTATACG      60

GGGAATAAT                                                              69

(2) INFORMATION FOR SEQ ID NO:9:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 65 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear


        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATTATTCCCC GTATAAGGAT CCCCCGGGAA GCTTTTAAGA CCGGATATTA AAAAACATAA      60

AGTGT                                                                  65

(2) INFORMATION FOR SEQ ID NO:10:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 72 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear


        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GATCAGAAAA ACTAGCTAGC TAGTACGTAG TTAACGTCGA CCTGCAGAAG CTTCTAGCTA      60

GCTAGTTTTT AT                                                          72

(2) INFORMATION FOR SEQ ID NO:11:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 72 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear


        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCTATAAAA ACTAGCTAGC TAGAAGCTTC TGCAGGTCGA CGTTAACTAC GTACTAGCTA      60

GCTAGTTTTT CT                                                          72
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 3659 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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GATATCTGTG GTCTATATAT ACTACACCCT ACCGATATTA ACCAACGAGT TTCTCACAAG      60

AAAACTTGTT TAGTAGATAG AGATTCTTTG ATTGTGTTTA AAAGAAGTAC CAGTAAAAAG     120

TGTGGCATAT GCATAGAAGA AATAAACAAA AAACATATTT CCGAACAGTA TTTTGGAATT     180

CTCCCAAGTT GTAAACATAT TTTTTGCCTA TCATGTATAA GACGTTGGGC AGATACTACC     240

AGAAATACAG ATACTGAAAA TACGTGTCCT GAATGTAGAA TAGTTTTTCC TTTCATAATA     300

CCCAGTAGGT ATTGGATAGA TAATAAATAT GATAAAAAAA TATTATATAA TAGATATAAG     360

AAAATGATTT TTACAAAAAT ACCTATAAGA ACAATAAAAA TATAATTACA TTTACGGAAA     420

ATAGCTGGTT TTAGTTTACC AACTTAGAGT AATTATCATA TTGAATCTAT ATTGTTTTTT     480

AGTTATATAA AAACATGATT AGCCCCCAAT CGGATGAAAA TATAAAGAT GTTGAGAATT      540

TCGAATACAA CAAAAGAGG AATCGTACGT TGTCCATATC CAAACATATA AATAAAAATT      600

CAAAAGTAGT ATTATACTGG ATGTTTAGAG ATCAACGTGT ACAAGATAAT TGGGCTTTAA     660

TTTACGCACA ACGATTAGCG TTAAAACTCA AAATACCTCT AAGAATATGC TTTTGTGTCG     720

TGCCAAAATT TCACACTACT ACTTCTAGAC ACTTTATGTT TTTAATATCC GGTCTTAAAG     780

AAGTCGCGGA AGAATGTAAA AGACTATGTA TAGGGTTTTC ATTGATATAT GGCGTACCAA     840

AAGTAATAAT TCCGTGTATA GTAAAAAAAT ACAGAGTCGG AGTAATCATA ACGGATTTCT     900

TTCCATTACG TGTTCCCGAA AGATTAATGA AACAGACTGT AATATCTCTT CCAGATAACA     960

TACCTTTTAT ACAAGTAGAC GCTCATAATA TAGTACCTTG TTGGGAAGCT TCTGATAAAG    1020

AAGAATACGG TGCACGAACT TTAAGAAAAA AGATATTTGA TAAATTATAT GAATATATGA    1080

CAGAATTTCC TGTTGTTCGT AAACATCCAT ACGGTCCATT TTCTATATCT ATTGCAAAAC    1140

CCAAAAATAT ATCATTAGAC AAGACGGTAT TACCCGTAAA ATGGGCAACG CCTGGAACAA    1200

AAGCTGGAAT AATTGTTTTA AAAGAATTTA TAAAAAACAG ATTACCGTCA TACGACGCGG    1260

ATCATAACAA TCCTACGTGT GACGCTTTGA GTAACTTATC TCCGTGGCTA CATTTTGGTC    1320

ATGTATCCGC ACAACGTGTT GCCTTAGAAG TATTAAAATG TATACGAGAA AGCAAAAAAA    1380

ACGTTGAAAC GTTTATAGAT GAAATAATTG TAAGAAGAGA ACTATCGGAT AATTTTTGTT    1440

ACTATAACAA ACATTATGAT AGTATCCAGT CTACTCATTC ATGGGTTAGA AAAACATTAG    1500

AAGATCACAT TAATGATCCT AGAAAGTATA TATATTCCAT TAAACAACTC GAAAAAGCGG    1560

AAACTCATGA TCCTCTATGG AACGCGTCAC AAATGCAGAT GGTGAGAGAA GGAAAAATGC    1620

ATAGTTTTTT ACGAATGTAT TGGGCTAAGA AGATACTTGA ATGGACTAGA ACACCTGAAG    1680
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ACGCTTTGAG TTATAGTATC TATTTGAACA ACAAGTACGA ACTAGACGGC ACGGATCCTA    1740

ACGGATACGT AGGTTGTATG TGGTCTATTT GCGGATTACA CGATAGAGCG TGGAAAGCAA    1800

GACCGATATT TGGAAAGATA AGATATATGA ATTATGAGAG TTCTAAGAAG AAATTTGATG    1860

TTGCTGTATT TATACAGAAA TACAATTAAG ATAAATAATA TACAGCATTG TAACCATCGT    1920

CATCCGTTAT ACGGGAATA ATATTACCAT ACAGTATTAT TAAATTTTCT TACGAAGAAT    1980

ATAGATCGGT ATTTATCGTT AGTTTATTTT ACATTTATTA ATTAAACATG TCTACTATTA    2040

CCTGTTATGG AAATGACAAA TTTAGTTATA TAATTTATGA TAAAATTAAG ATAATAATAA    2100

TGAAATCAAA TAATTATGTA AATGCTACTA GATTATGTGA ATTACGAGGA AGAAAGTTTA    2160

CGAACTGGAA AAAATTAAGT GAATCTAAAA TATTAGTCGA TAATGTAAAA AAAATAAATG    2220

ATAAAACTAA CCAGTTAAAA ACGGATATGA TTATATACGT TAAGGATATT GATCATAAAG    2280

GAAGAGATAC TTGCGGTTAC TATGTACACC AAGATCTGGT ATCTTCTATA TCAAATTGGA    2340

TATCTCCGTT ATTCGCCGTT AAGGTAAATA AAATTATTAA CTATTATATA TGTAATGAAT    2400

ATGATATACG ACTTAGCGAA ATGGAATCTG ATATGACAGA AGTAATAGAT GTAGTTGATA    2460

AATTAGTAGG AGGATACAAT GATGAAATAG CAGAAATAAT ATATTTGTTT AATAAATTTA    2520

TAGAAAAATA TATTGCTAAC ATATCGTTAT CAACTGAATT ATCTAGTATA TTAAATAATT    2580

TTATAAATTT TATAAATTTT AATAAAAAAT ACAATAACGA CATAAAGATA TTTAATCTTT    2640

AATTCTTGAT CTGAAAAACA CATCTATAAA ACTAGATAAA AAGTTATTCG ATAAAGATAA    2700

TAATGAATCG AACGATGAAA AATTGGAAAC AGAAGTTGAT AAGCTAATTT TTTTCATCTA    2760

AATAGTATTA TTTTATTGAA GTACGAAGTT TTACGTTAGA TAAATAATAA AGGTCGATTT    2820

TTACTTTGTT AAATATCAAA TATGTCATTA TCTGATAAAG ATACAAAAAC ACACGGTGAT    2880

TATCAACCAT CTAACGAACA GATATTACAA AAAATACGTC GGACTATGGA AAACGAAGCT    2940

GATAGCCTCA ATAGAAGAAG CATTAAAGAA ATTGTTGTAG ATGTTATGAA GAATTGGGAT    3000

CATCCTCAAC GAAGAAATAG ATAAAGTTCT AAACTGGAAA AATGATACAT TAAACGATTT    3060

AGATCATCTA AATACAGATG ATAATATTAA GGAAATCATA CAATGTCTGA TTAGAGAATT    3120

TGCGTTTAAA AAGATCAATT CTATTATGTA TAGTTATGCT ATGGTAAAAC TCAATTCAGA    3180

TAACGAACAT TGAAAGATAA AATTAAGGAT TATTTTATAG AAACTATTCT TAAAGACAAA    3240

CGTGGTTATA AACAAAGCC ATTACCCGGA TTGGAAACTA AAATACTAGA TAGTATTATA    3300

AGATTTTAAA AACATAAAAT TAATAGGTTT TTATAGATTG ACTTATTATA TACAATATGG    3360

ATAAAAGATA TATATCAACT AGAAAGTTGA ATGACGGATT CTTAATTTTA TATTATGATT    3420

CAATAGAAAT TATTGTCATG TCGTGTAATC ATTTTATAAA TATATCAGCG TTACTAGCTA    3480

AGAAAAACAA GGACTTTAAT GAATGGCTAA AGATAGAATC ATTTAGAGAA ATAATAGATA    3540

CTTTAGATAA AATTAATTAC GATCTAGGAC AACGATATTG TGAAGAACTT ACGGCGCATC    3600

ACATTCCAGT GTAATTATTG AGGTCAAAGC TAGTAACTTA ATAGATGACA GGACAGCTG     3659
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(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 70 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AATTAACCCG GGATCCAAGC TTCTAGCTAG CTAATTTTTA TAGCGGCCGC TATAATCGTT   60

AACTTATTAG   70

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 67 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTAGCTAGAA GCTTGGATCC CGGGTTAATT AATTAATAAA AAGCGGCCGC GTTAAAGTAG   60

AAAAATG   67

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTTACATATG TACAGAATCT GATCATAG   28

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCTAGAATTC TCTTAGTTTT TATAGTTG   28

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 18 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AAGGTAGTAC TGGCGTCC   18

    (2) INFORMATION FOR SEQ ID NO:18:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 54 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTATCGCGAT ATCCGTTAAG TTTGTATCGT AATATGTTCC CTCACAATCC ACGA   54

    (2) INFORMATION FOR SEQ ID NO:19:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 26 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TAAAAGCTTT TAATTAATTA GTCATC   26

    (2) INFORMATION FOR SEQ ID NO:20:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 26 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TAACCCGGGC GATACAAACT TAACGG   26

    (2) INFORMATION FOR SEQ ID NO:21:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 72 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GATCCCGGTA CCTCTAATGC TGATCATCCG AACCGCGCTG ACACTGAGCT GTACAAACCT   60

GCAAGATCAA AC   72

(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGACGCCGGT CCGGTTGTTG GCATC   25

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 112 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTGAGTACTT CATGGAGGTG GCCGACCTCA ACTCTCCCCT GAAGATTGCA GGAGCATTTG   60

GCTTCAAAGA CATAATCCGG GCTATAAGGA GGATCGTTTT AACTGTCATA TC   112

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 31 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TTAGGATCCT CATATTTTTG TAGTGGCTCT C   31

(2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 42 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TACACACTGC AGAGCAATGG GAACCTCAAG TTCGATCAGA TG   42

(2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 39 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GAAACACGAG CTCTCCCCCA ACGCTGAGGC TTGTGATAG   39

(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 155 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGAAGAGCTC GTGTTTCAAA CAAGCGTCCA AGGCCTTGTA CTGGGCGCCA CCATCTACTT   60

TATAGGCTTT GATGGGACTA CGGTAATCAC CAGAGCTGTA GCCGCAGATA ATGGGCTGAC   120

GGCCGGCACC GACAATCTTA TGCCATTCAA TCTTG   155

(2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 155 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CCACCATGGA TCGTCACTGC TAGGCTCCCA CTTGCCGACC ATGACATCTG ATCCCCTGCC   60

TGACCACCAC TTTTGGAGGT CACTACCTCC AGTTTGATGG ATGTGATTGG CTGGGTTATC   120

TCATTGGTTG GAATGACAAG ATTGAATGGC ATAAG   155

(2) INFORMATION FOR SEQ ID NO:29:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGGAGAGCTC GTGTTTCAAA CAAGCG   26

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CCACCATGGA TCGTCACTGC   20

(2) INFORMATION FOR SEQ ID NO:31:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 17 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AACATATTTC CGAACAG   17

(2) INFORMATION FOR SEQ ID NO:32:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 57 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCCAAGCTTT CGCGACCCGG GTTTTTATTA GCTAATTAGC AATATAGATT CAATATG   57

(2) INFORMATION FOR SEQ ID NO:33:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 62 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ATCAAGCTTG GATCCCTCGA GTTTTTATTG ACTAGTTAAT CATAAGATAA ATAATATACA   60

GC   62

(2) INFORMATION FOR SEQ ID NO:34:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 17 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GATATAGAAG ATACCAG   17

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WHAT IS CLAIMED IS:

1. A recombinant poxvirus containing therein DNA from infectious bursal disease virus in a nonessential region of the poxvirus genome.

2. A recombinant poxvirus as in claim 1 wherein said DNA codes for and expresses an infectious bursal disease virus structural protein.

2. A recombinant poxvirus as in claim 2 wherein said structural protein is VP2.

3. A recombinant poxvirus as in claim 2 wherein said structural protein is VP3.

4. A recombinant poxvirus as in claim 2 wherein said structural protein is polyprotein VP2, VP3, VP4.

5. A recombinant poxvirus as in claim 1 wherein the poxvirus is an avipox virus.

6. A recombinant avipox virus as in claim 6 wherein the avipox virus is fowlpox virus.

7. A recombinant fowlpox virus as in claim 7 which is selected from the group consisting of vFP115, vFP116, vFP147, vFP151, vFP186 and vFP209.

8. A vaccine for inducing an immunological response in a host animal inoculated with said vaccine, said vaccine comprising a carrier and a recombinant poxvirus containing, in a nonessential region thereof, DNA from infectious bursal disease virus.

9. A vaccine as in claim 9 wherein said DNA codes for and expresses an infectious bursal disease virus structural protein.

10. A vaccine as in claim 10 wherein said structural protein is VP2.

11. A vaccine as in claim 10 wherein said structural protein is VP3.

12. A vaccine as in claim 10 wherein said structural protein is polyprotein VP2, VP3, VP4.

13. A vaccine as in claim 9 wherein the poxvirus is an avipox virus.

14.　A vaccine as in claim 14 wherein the avipox virus is fowlpox virus.

15.　A vaccine as in claim 14 wherein the host animal is a chicken.

AMENDED CLAIMS

[received by the International Bureau on 14 December 1992 (14.12.92);
second claim 2 renumbered as claim 3,
original claims 3-15 renumbered as claims 4-16;
claim 1 unchanged (2 pages)]

1. A recombinant poxvirus containing therein DNA from infectious bursal disease virus in a nonessential region of the poxvirus genome.

5 2. A recombination poxvirus as in claim 1 wherein said DNA codes for and expresses an infectious bursal disease virus structural protein.

3. A recombinant poxvirus as in claim 2 wherein said structural protein is VP2.

10 4. A recombinant poxvirus as in claim 2 wherein said structural protein is VP3.

5. A recombinant poxvirus as in claim 2 wherein said structural protein is polyprotein VP2, VP3, VP4.

15 6. A recombinant poxvirus as in claim 1 wherein the poxvirus is an avipox virus.

7. A recombinant avipox virus as in claim 6 wherein the avipox virus is fowlpox virus.

8. A recombinant fowlpox virus as in claim 7
20 which is selected from the group consisting of vFP115, vFP116, vFP147, vFP151, vFP186 and vFP209.

9. A vaccine for inducing an immunological response in a host animal inoculated with said vaccine, said vaccine comprising a carrier and a recombinant
25 poxvirus containing, in a nonessential region thereof, DNA from infectious bursal disease virus.

10. A vaccine as in claim 9 wherein said DNA codes for and expresses an infectious bursal disease virus structural protein.

30 11. A vaccine as in claim 10 wherein said structural protein is VP2.

12. A vaccine as in claim 10 wherein said structural protein is VP3.

13. A vaccine as in claim 10 wherein said
35 structural protein is polyprotein VP2, VP3, VP4.

14. A vaccine as in claim 9 wherein the

15. A vaccine as in claim 14 wherein the
avipox virus is fowlpox virus.

16. A vaccine as in claim 14 wherein the host
animal is a chicken.

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           10        20        30        40        50        60        70        80        90       100
GATATCTGTGGTCTATATACTACACCCTACCGATATTAACCAACGAGTTTCTCACAAGAGAAAACTTGTTTAGTAGATAGAGATTCTTTGATTGTGTTTA

          110       120       130       140       150       160       170       180       190       200
AAAGAAGTACCAGTAGAGAAAAAGTGTGGCATATGCATAGAGAGAAATAAACAAAAAACATATTTCCGAACAGTATTTGGAATTCTCCCAAGTTGTAAACATAT

          210       220       230       240       250       260       270       280       290       300
TTTTGCCTATCATGTATAAGACGTTGGGCAGATACTACCAGAAATACAGATACTGAAATACGTGTCCTGAATGTAGAAATAGTTTTTCCTTTCATAAATA

          310       320       330       340       350       360       370       380       390       400
CCCAGTAGGTATTGGATAGAGATAATAAATATTATTATATAATAGATAAAATGATTTTTACAAAAAATATTATAAGAAAAATAACCTATAAGAACAATAAAA

          410       420       430       440       450       460       470       480       490       500
ATATAATTACATTTACGGAAAATAGCTGGTTTTTAGTTTACCAACTTAGAGTAATTATCATATTGAATCTATATTGTTTTTAGTTATATAAAAACATGAT

          510       520       530       540       550       560       570       580       590       600
TAGCCCCCAATCGGATGAGAAAATATAAAAAGATGTTGAGAATTTCGAATACAACAAAAAGAGGAATCGTGTCCATATCCAAACATATAAAATAAAAAT

          610       620       630       640       650       660       670       680       690       700
TCAAAAGTAGTATTATACTGATGTTTAGAGATCAACGTGTACAAGATAATTGGGCTTTAATTTACGCACAACGATTAGGCGTTAAAACTCAAAATACCTC

          710       720       730       740       750       760       770       780       790       800
TAAGAATATGCTTTGTCGTGTGCCAAATTTCCACACTTTATGTTTTTAATATCCGGTCTTAAAGAAGTCGCGGAAGAATGTA

          810       820       830       840       850       860       870       880       890       900
AAAGACTATGTATAGGGGTTTTCATTGATATATGGCGTACCAAAAGTAATAATTCCGTGTATAGTAGTAAAAAAATACAGAGTCGGAGTAATCATAACGGATTT

          910       920       930       940       950       960       970       980       990      1000
CTTTCCATTACGTGTTCCCGAAAGATTGTAATGAAACAGTAATATCTCTTCCAGATAACAACATACCTTTATACAAGTAGAACGCTCATAATATAGTACCT

         1010      1020      1030      1040      1050      1060      1070      1080      1090      1100
TGTTGGGAAGCTTCTGATAAAGAAGAAATACGGTGCACGAACTTTAAGAAAAAGATATTTGATAAATTATATGAATATGACAGAATTTCCTGTTGTTC

         1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
GTAAACATCCATACGGTCCATTTTCTATATCTATTGCAAAACCCAAAAAATATATCATTAGACAAGAGCGGTATTACCCGTAAAATGGGCAACGCCTGGAAC

         1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
AAAAGCTGGAATAATTGTTTTAAAGAATTTATAAAAAAACAGATTACGTCATAACGACGCGGATCATAACAATCCTACGTGTGACGCTTTGAGTAACTTA
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FIG. 1A

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            1310      1320      1330      1340      1350      1360      1370      1380      1390      1400
TCTCCGTGGCTACACATTTTGGGTCATGTGTATCCGCACAACGTGTGTTGCCTTAGAAGTATTAAAATGTATACGAGGAAAGCAAAAAAAACGTTGAAACGTTTATAG

            1410      1420      1430      1440      1450      1460      1470      1480      1490      1500
ATGAAATAATTGTAAGAGAGAACTATCGGATAATTTTGTTACTATAACAAACATTATGATAGTAGTATCCAGTCTACTCATTCATGGGTTAGAAAAACATT

            1510      1520      1530      1540      1550      1560      1570      1580      1590      1600
AGAAGATCACATTAATGATCCTAGAAAGTATATATATTCCATTAAACAACTCGAAAAAGCGGAAACTCATGATCCTCTATGGAACGCGTCACAAATGCAG

            1610      1620      1630      1640      1650      1660      1670      1680      1690      1700
ATGGGTGAGAGGAGGAAAAATGCATAGTTTTTTTACGAATGTATTGGGCTAAGAAGAGATACTTGAATGGAACACCTGAGAGACGCCTTTGAGTTATAGTA

            1710      1720      1730      1740      1750      1760      1770      1780      1790      1800
TCTATTTGAACAACAAGTACGAACTAGACGGGCACGGATCCTAACGGATACGTAGGTTGTATGTGGTCTATTTGCGGATTACACGATAGAGCGTGGAAAGC

            1810      1820      1830      1840      1850      1860      1870      1880      1890      1900
AAGACCGATATTTGGAAAGATAAGATATATGAATTATGAGAGTTCTAAGAAGAAATTTGATGTTGCTGTATTTATACAGAAATACAATTAAGATAAATAA

            1910      1920      1930      1940      1950      1960      1970      1980      1990      2000
TATACAGCATTGTAACCATCGTCATCCGTTATACGGGGAATAAATATTACCATACAGTATTATTAAATTTCTTACGGAAGAATATAGATCGGTATTTATCG

            2010      2020      2030      2040      2050      2060      2070      2080      2090      2100
TTAGTTTATTTTACATTTTATTAATTAAACATGTCTACTATTACCTGTTATGGAAATGACAAATTTAGTTATATAAATTATGATAAAATTAAGATAATAAT

            2110      2120      2130      2140      2150      2160      2170      2180      2190      2200
AATGAAATCAAATAATTATGTAAATGCTACTAGAATTATGTGTGAATTAACGGAGGAGAAGAAGTTTACGAACTGGAAAAAATTAAGTGAATCTAAAATATTAGTC

            2210      2220      2230      2240      2250      2260      2270      2280      2290      2300
GATAATGTAAAAAAAATAAAATGATAAAACGGATAAAACTAACCAGTTAAAACAACGGTTAAGGGATATTGATCATAAAGGAAGAGATACTTGCGGTT

            2310      2320      2330      2340      2350      2360      2370      2380      2390      2400
ACTATGTACACCAAGATCTGGTATCTTCTATATCAAATTGGATATCTCCGTTATTCGCCGTTAAGGTAAATAAAATAAAAATTATTAACTATTATATGTAATGA

            2410      2420      2430      2440      2450      2460      2470      2480      2490      2500
ATATGATATACGACTTAGCGAAATGGAATCTGATATGACAGAAGTAATAGATGTAGTTGATAAAAATTAGTAGGAGGATACAATGATGAAATAGCAGAAATA
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FIG. 1B

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        2510      2520      2530      2540      2550      2560      2570      2580      2590      2600
ATATATTTGTTTAATAAATTTATAGAAAAATATATTGCTAACACATATCGTTATCAACTGAATTATCTAGTATTAAAATAATTTTATAAATT

        2610      2620      2630      2640      2650      2660      2670      2680      2690      2700
TTAATAAAAATACAATAACGACACATAAAGATATTTAATTCTTGATCTGAAAAACACATCTATAAAACTAGATAAAAAGTTATTCGATAAAGAT

        2710      2720      2730      2740      2750      2760      2770      2780      2790      2800
AATAATGAATCGAACGATGAAAAATTGGAAACAGAAGTTGATAAGCTAATTTTTTCATCTAAATAGTATTATTGAAGTACGAAGTTTACGTTA

        2810      2820      2830      2840      2850      2860      2870      2880      2890      2900
GATAAAATAATAAAGGTCGATTTTTTACTTTGTTAAATATCAAATAGTCATTATTCTGATAAAGATACAAAAACACGGTGATTATCAACCATCTAACGAA

        2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
CAGATATTACAAAAAATACGTCGGACTATGGAAAACGAAGCTCAATAGAGAAGCATTAAAGAAATTGTTGTAGATGTTATGAAGAATTGGG

        3010      3020      3030      3040      3050      3060      3070      3080      3090      3100
ATCATCCTCAACGAAGAATAGATAAGTTCTAAACTGGAAAAATGATACATTAAACGATTAGATCATCTAAATACAGATGATAATATTAAGGAAATCA

        3110      3120      3130      3140      3150      3160      3170      3180      3190      3200
TACAATGTCTGATTAGAGAATTTGCGTTTAAAAAGATCAATTCTATTATGTATAGTTATGCTATGGTAAAACTCAATTCAGATAACGAACATTGAAAGAT

        3210      3220      3230      3240      3250      3260      3270      3280      3290      3300
AAAATTAAGGATTATTTTATAGAAACTATTCTTAAAGAGACAAACGTGGTTATAAAACAAAAGCCATTACCCGGATTGGAAACTAAAAATACTAGATAGTATTA

        3310      3320      3330      3340      3350      3360      3370      3380      3390      3400
TAAGATTTTAAAAAACATAAAAATTAATAGGTTTTTTATAGATTGACTTATTATATACAATATGGGATAAAAGATAAAGTTGAATGACGGA

        3410      3420      3430      3440      3450      3460      3470      3480      3490      3500
TTCTTAATTTTATATTATGATTCAATAGAAATTATTGTCATGTCGTGTAATCATTTTATAAATATATCAGGGTTACTAGCTAAGAAAAACAAGGACTTTA

        3510      3520      3530      3540      3550      3560      3570      3580      3590      3600
ATGAATGGCTAAAGATAGAATCATTTAGAGAGAAATAATAGTACTTTAGATAAAATTAATTACGATCTAGGACAACGATATTGTGAAGAACTTACGGCGCA

        3610      3620      3630      3640      3650      3660
TCACATTCCAGTGTGTAATTATTGGAGGTCAAAGCTAGTAACTTTAATAGATGACAGGACAGCTG
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FIG. 1C

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   1 GATATCTGTG GTCTATATAT ACTACACCCT ACGGATATTA ACCAACGAGT TTCTCACAAG
  61 AAAACTTGTT TAGTAGATAG AGATTCTTTG ATTGTGTTTA AAAGAAGTAC CAGTAAAAAG
 121 TGTGGCATAT GCATAGAAGA AATAAACAAA AAACATATTT CCGAACAGTA TTTTGGAATT
 181 CTCCCAAGTT GTAAACATAT TTTTTGCCTA TCATGTATAA GACGTTGGGC AGATACTACC
 241 AGAAATACAG ATACTGAAAA TACGTGTCCT GAATGTAGAA TAGTTTTTCC TTTCATAATA
 301 CCCAGTAGGT ATTGGATAGA TAATAAATAT GATAAAAAAA TATTATATAA TAGATATAAG
 361 AAAATGATTT TTACAAAAAT ACCTATAAGA ACAATAAAAA TATAATTACA TTTACGGAAA
 421 ATAGCTGGTT TTAGTTTACC AACTTAGAGT AATTATCATA TTGAATCTAT ATTGTTTTTT
 481 AGTTATATAA AAACATGATT AGCCCCCAAT CGGATGAAAA TATAAAGAT GTTGAGAATT
 541 TCGAATACAA CAAAAAGAGG AATCGTACGT TGTCCATATC CAAACATATA AATAAAAATT
 601 CAAAAGTAGT ATTATACTGG ATGTTTAGAG ATCAACGTGT ACAAGATAAT TGGGCTTTAA
 661 TTTACGCACA ACGATTAGCG TTAAAACTCA AAATACCTCT AAGAATATGC TTTTGTGTCG
 721 TGCCAAAATT TCACACTACT ACTTCTAGAC ACTTTATGTT TTAATATCC GGTCTTAAAG
 781 AAGTCGCGGA AGAATGTAAA AGACTATGTA TAGGGTTTTC ATTGATATAT GGCGTACCAA
 841 AAGTAATAAT TCCGTGTATA GTAAAAAAAT ACAGAGTCGG AGTAATCATA ACGGATTTCT
 901 TTCCATTACG TGTTCCCGAA AGATTAATGA AACAGACTGT AATATCTCTT CCAGATAACA
 961 TACCTTTTAT ACAAGTAGAC GCTCATAATA TAGTACCTTG TTGGGAAGCT TCTGATAAAG
1021 AAGAATACGG TGCACGAACT TTAAGAAAAA AGATATTTGA TAAATTATAT GAATATATGA
1081 CAGAATTTCC TGTTGTTCGT AAACATCCAT ACGGTCCATT TTCTATATCT ATTGCAAAAC
1141 CCAAAAATAT ATCATTAGAC AAGACGGTAT TACCCGTAAA ATGGGCAACG CCTGGAACAA
1201 AAGCTGGAAT AATTGTTTTA AAAGAATTTA TAAAAAACAG ATTACCGTCA TACGACGCGG
1261 ATCATAACAA TCCTACGTGT GACGCTTTGA GTAACTTATC TCCGTGGCTA CATTTTGGTC
1321 ATGTATCCGC ACAACGTGTT GCCTTAGAAG TATTAAAATG TATACGAGAA AGCAAAAAAA
1381 ACGTTGAAAC GTTTATAGAT GAAATAATTG TAAGAAGAGA ACTATCGGAT AATTTTTGTT
1441 ACTATAACAA ACATTATGAT AGTATCCAGT CTACTCATTC ATGGGTTAGA AAAACATTAG
1501 AAGATCACAT TAATGATCCT AGAAAGTATA TATATTCCAT TAAACAACTC GAAAAAGCGG
1561 AAACTCATGA TCCTCTATGG AACGCGTCAC AAATGCAGAT GGTGAGAGAA GGAAAAATGC
1621 ATAGTTTTTT ACGAATGTAT TGGGCTAAGA AGATACTTGA ATGGACTAGA ACACCTGAAG
1681 ACGCTTTGAG TTATAGTATC TATTTGAACA ACAAGTACGA ACTAGACGGC ACGGATCCTA
1741 ACGGATACGT AGGTTGTATG TGGTCTATTT GCGGATTACA CGATAGAGCG TGGAAAGCAA
1801 GACCGATATT TGGAAAGATA AGATATATGA ATTATGAGAG TTCTAAGAAG AAATTTGATG
1861 TTGCTGTATT TATACAGAAA TACAATTAAG ATAAATAATA TACAGCATTG TAACCATCGT
1921 CATCCGTTAT ACGGGGAATA ATATTACCAT ACAGTATTAT TAAATTTTCT TACGAAGAAT
1981 ATAGATCGGT ATTTATCGTT AGTTTATTTT ACATTTATTA ATTAAACATG TCTACTATTA
2041 CCTGTTATGG AAATGACAAA TTTAGTTATA TAATTTATGA TAAAATTAAG ATAATAATAA
2101 TGAAATCAAA TAATTATGTA AATGCTACTA GATTATGTGA ATTACGAGGA AGAAAGTTTA
2161 CGAACTGGAA AAAATTAAGT GAATCTAAAA TATTAGTCGA TAATGTAAAA AAAATAAATG
2221 ATAAAACTAA CCAGTTAAAA ACGGATATGA TTATATACGT TAAGGATATT GATCATAAAG
2281 GAAGAGATAC TTGCGGTTAC TATGTACACC AAGATCTGGT ATCTTCTATA TCAAATTGGA
2341 TATCTCCGTT ATTCGCCGTT AAGGTAAATA AAATTATTAA CTATTATATA TGTAATGAAT
2401 ATGATATACG ACTTAGCGAA ATGGAATCTG ATATGACAGA AGTAATAGAT GTAGTTGATA
2461 AATTAGTAGG AGGATACAAT GATGAAATAG CAGAAATAAT ATATTTGTTT AATAAATTTA
2521 TAGAAAAATA TATTGCTAAC ATATCGTTAT CAACTGAATT ATCTAGTATA TTAAATAATT
2581 TTATAAATTT TATAAATTTT AATAAAAAAT ACAATAACGA CATAAAGATA TTTAATCTTT
2641 AATTCTTGAT CTGAAAAACA CATCTATAAA ACTAGATAAA AAGTTATTCG ATAAAGATAA
2701 TAATGAATCG AACGATGAAA AATTGGAAAC AGAAGTTGAT AAGCTAATTT TTTTCATCTA
2761 AATAGTATTA TTTTATTGAA GTACGAAGTT TTACGTTAGA TAAATAATAA AGGTCGATTT
2821 TTACTTTGTT AAAATATCAAA TATGTCATTA TCTGATAAAG ATACAAAAC ACACGGTGAT
2881 TATCAACCAT CTAACGAACA GATATTACAA AAAATACGTC GGACTATGGA AAACGAAGCT
2941 GATAGCCTCA ATAGAAGAAG CATTAAAGAA ATTGTTGTAG ATGTTATGAA GAATTGGGAT
3001 CATCCTCAAC GAAGAAATAG ATAAAGTTCT AAACTGGAAA AATGATACAT TAAACGATTT
3061 AGATCATCTA AATACAGATG ATAATATTAA GGAAATCATA CAATGTCTGA TTAGAGAATT
3121 TGCGTTTAAA AAGATCAATT CTATTATGTA TAGTTATGCT ATGGTAAAAC TCAATTCAGA
3181 TAACGAACAT TGAAAGATAA AATTAAGGAT TATTTTATAG AAACTATTCT TAAAGACAAA
3241 CGTGGTTATA AACAAAAGCC ATTACCCGGA TTGGAAACTA AAATACTAGA TAGTATTATA
3301 AGATTTTAAA AACATAAAAT TAATAGGTTT TTATAGATTG ACTTATTATA TACAATATGG
3361 ATAAAAGATA TATATCAACT AGAAAGTTGA ATGACGGATT CTTAATTTTA TATTATGATT
3421 CAATAGAAAT TATTGTCATG TCGTGTAATC ATTTTATAAA TATATCAGCG TTACTAGCTA
3481 AGAAAAACAA GGACTTTAAT GAATGGCTAA AGATAGAATC ATTTAGAGAA ATAATAGATA
3541 CTTTAGATAA AATTAATTAC GATCTAGGAC AACGATATTG TGAAGAACTT ACGGCGCATC
3601 ACATTCCAGT GTAATTATTG AGGTCAAAGC TAGTAACTTA ATAGATGACA GGACAGCTG
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FIG. 2

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5)   :C12N 7/00; C12P 21/02; A61K 39/12
US CL   :435/69.3, 235.1; 424/89

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.3, 235.1; 424/89

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, search terms: poxvirus, infectious bursal disease virus, VP2, VP3, VP4

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Azad et al. "Vaccines 90", published 1990 by Cold Spring Harbor Laboratory Press, CSH (NY), "Full Protection Against an Immunodepressive Viral Disease by a Recombinant Antigen Produced in Yeast", pages 59-62, entire document.	1, 2, 5, 6, 8
X,P	Archives of Virology, Volume 120, issued November 1991, C.D. Bayliss et al., "A Recombinant Fowlpox Virus that Expresses the VP2 Antigen of Infectious Bursal Disease Virus Induces Protection Against Mortality Caused by the Virus", pages 193-205, entire document.	1, 2, 5, 6, 8
Y	Virology, volume 161, issued October 1987, A.A. Azad et al., "Deletion Mapping and Expression in Escherichia coli of the Large Genomic Segment of a Birnavirus", pages 145-152, entire document.	1, 2, 5, 6, 8
Y	Nucleic Acids Research, Volume 14, No. 12, issued September 1986, P.J. Hudson et al., "Genomic Structure of the Large RNA Segment of Infectious Bursal Disease Virus", pages 5001-5012, entire document.	1, 2, 5, 6, 8

X	Further documents are listed in the continuation of Box C.		See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 OCTOBER 1992	22 OCT 1992

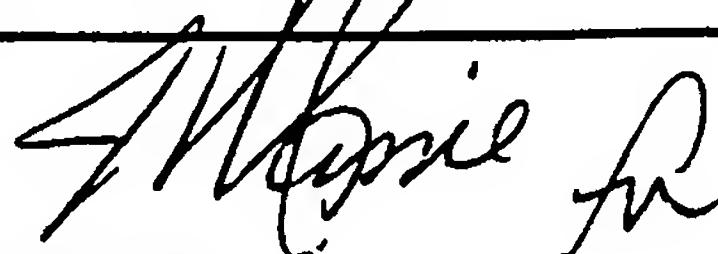| Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer JOAN ELLIS
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of General Virology, Volume 69, issued November 1988, F.S.B. Kibenge et al., "Biochemistry and Immunology of Infectious Bursal Disease Virus", pages 1757-1775, see especially page 1769.	1, 2, 5, 6, 8
Y	Journal of Virology, Volume 64, No. 4, issued April 1990, J. Taylor et al., "Newcastle disease Viurs Fusion Protein Expressed in a Fowlpox Virus Recombinant Confers Protection in Chickens", pages 1441-1450, entire document.	1, 2, 5, 6, 8
Y	Vaccine, Volume 6, issued December 1988, J. Taylor et al. "Protective Immunity Against Avian Influenza Induced by a Fowlpox Virus Recombinant", pages 504-508, entire document.	1, 2, 5, 6, 8

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

Two claims were labelled as claim 2, therefore, it was unclear what applicants intended by the second claim 2. In addition, it was not clear on which claim 2 claims 3 and 4 depended. Claims 7, 9, 10, and 14 all were dependent upon themselves. Accordingly, neither they, or the claims dependent upon them, were searchable.